REMARKS

Status of the Claims

Claims 1, 4-7, and 9-15 are pending in the present application. Claims 9-15 are withdrawn as directed to a non-elected invention. Claim 1 is amended to incorporate the elements of claims 3 and 8, now canceled. Claim 2 is also canceled. No new matter is added by way of this amendment. Reconsideration is respectfully requested.

Specification

The Examiner requests that Applicants review the present application for all possible minor errors, see Office Action, page 4. If Applicants become aware of any minor errors in the instant application, Applicants will amend the specification accordingly.

Rejection Under 35 U.S.C. § 112, First Paragraph

Written Description

Claims 1-8 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement, *see* Office Action, pages 4-7. Applicants respectfully traverse.

Basis for the rejection

According to the Examiner, the specification fails to disclose how variant sequences can be fused and result in a DNA, which encodes a protein that retains glucose dehydrogenase activity and an electron transfer ability. According to the Examiner, the instant specification fails to disclose a correlation between structure and function. The Examiner further states that the specification fails to describe those amino acid residues, which are responsible for catalytic activity.

Standard for compliance with the written description requirement

To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. *See, e.g., Moba, B.V. v. Diamond Automation,*

Inc., 325 F.3d 1306, 1319, 66 USPQ2d 1429, 1438 (Fed. Cir. 2003); *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116.

The descriptive text in the specification needed to meet the written description requirement, however, varies with the nature and scope of the invention at issue, and with the scientific and technologic knowledge already in existence. The law must be applied to each invention that enters the patent process, for each patented advance is novel in relation to the state of the science. Since the law is applied to each invention in view of the state of relevant knowledge, its application will vary with differences in the state of knowledge in the field and differences in the predictability of the science, *see Falkner v. Inglis*, 448 F.3d 1357, 79 USPQ2d 1001 (Fed. Cir. 2006).

The court has also voiced its approval of the "Written Description Guidelines" to aid in determining whether or not a claimed genus complies with the written description requirement. *See Carnegie Mellon University v. Hoffmann-LaRoche*, 541 F. 3d. 1115 (Fed Cir. 2008) and the "Written Description Training Materials", March 25, 2008, uspto.gov/web/medu/written.pdf, ("Guidelines").

Example 11B of the Guidelines, entitled "Art-Recognized Structure-Function Correlation Present" exemplifies a genus of nucleic acids that complies with the written description requirement. The claimed genus described in Example 11B encompasses nucleic acids that encode a polypeptide of a specified sequence and those that encode any polypeptide having at least 85% structural identity to the specified sequence, wherein the polypeptide, additionally, has activity Y. According to the Guidelines, the genus described in Example 11B complies with the written description requirement, *inter alia*, because the specification identifies two domains responsible for activity Y, *i.e.*, a binding domain and a catalytic domain. Accordingly, there is information about which nucleic acids can vary in the corresponding nucleotide sequence in the claimed genus of nucleic acids and still encode a polypeptide having activity Y. In view of the foregoing, the exemplified claim complies with the written description requirement.

The claimed invention

As amended, claim 1 is directed to a fusion protein of pyrroloquinoline quinone glucose dehydrogenase (PQQGDH) and a cytochrome, wherein the cytochrome has been fused to the C-terminal side of PQQGDH, and wherein the PQQGDH is either (a) or (b): (a) a protein

comprising an amino acid sequence represented by SEQ ID NO: 2; (b) a protein comprising an amino acid sequence in which one or more amino acid residues have been deleted, substituted or added in the amino acid sequence (a) and having a glucose dehydrogenase activity and an electron transfer ability.

The claims comply with the written description requirement

Applicants submit that at the time of the invention an ordinary artisan would have recognized which amino acids could have been deleted, substituted, or added in SEQ ID NO: 2 while concomitantly retaining glucose dehydrogenase activity and electron transfer capabilities. As described in the Examples of the originally filed application, SEQ ID NO: 2 is a fusion protein between PQQGDH from *Acinetobacter calcoaceticus* and a cytochrome c domain from *Comamonastes testosteroni*. Applicants submit that PQQGDH from *Acinetobacter calcoaceticus* is a well characterized enzyme. Further, amino acid residues had been identified at the time of filing, which have a role in, for example, substrate oxidation and/or substrate specificity, *see for example* Exhibit C11, *i.e.*, Igarashi *et al.*, "Engineering PQQ glucose dehydrogenase with improved substrate specificity site-directed mutagenesis studies on the active center of PQQ glucose dehydrogenase", *Biomol. Eng.*, April 2004, 21:81-9, including page 88, *enclosed*, and Exhibit 16, *i.e.*, Igarashi *et al.* "Molecular engineering of PQQGDH and its applications", *Arch. Biochem. Biophys.* August 1, 2004, 428:52-63, including page 58, Table 5, *enclosed.*

Moreover, a variety of PQQGDH mutants derived from *Acinetobacter calcoaceticus* PQQGDH were available in the art before or shortly after the priority date of the present invention, *see* Appendix and Exhibits A1-A3, B1-B10, and C1-C16, *enclosed.* The mutants described in the enclosed documents comprise various amino acid deletions, substitutions, or additions. Nevertheless, the mutants retain glucose dehydrogenase activity and electron transfer ability.

In view of the foregoing, Applicants submit that support for the instant claims is analogous to that described in Example 11B of the Guidelines. In particular, a correlation between structure and function of PQQGDH was known in the art at the time of filing. That is Exhibits C11 and C16, for example, describe amino acid residues correlated with catalytic activity and substrate specificity. Accordingly, contrary to the Examiner's assertions, there is

information about which nucleic acids can vary in the corresponding nucleotide sequence in the claimed genus of nucleic acids and still encode a polypeptide having the activity described in the claims.

As indicated in *Falkner, supra,* there is no *per se* requirement that information known in the art at the time of filing be repeated in a specification. As stated above, the descriptive text in the specification needed to meet the written description requirement varies with the nature and scope of the invention at issue, and with the scientific and technologic knowledge already in existence. Accordingly, the claims comply with the written description requirement and withdrawal of the rejection is respectfully requested.

Enablement

Claims 1-8 are also rejected under 35 U.S.C. § 112, first paragraph, because the specification allegedly fails to comply with the enablement requirement, *see* Office Action, pages 7-9. In particular, the Examiner states that it is not routine in the art to screen for multiple substitutions or multiple modifications of the fusion protein described in the present claims. The Examiner further states that positions within a protein's sequence wherein amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity is unpredictable.

The Federal Circuit has repeatedly held that "the specification must teach those skilled in the art how to make and use the full scope of the claimed invention without 'undue experimentation'." *In re Wright,* 999 F.2d 1557, 1561, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). Nevertheless, not everything necessary to practice the invention need be disclosed. In fact, what is well-known is best omitted. *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991). All that is necessary is that one skilled in the art be able to practice the claimed invention, given the level of knowledge and skill in the art, *see also* MPEP 2164.08.

As noted above, at the time of the invention an ordinary artisan would have recognized from art known at the time of filing which amino acids in SEQ ID NO: 2 could have been altered without a concomitant loss of glucose dehydrogenase activity and electron transfer ability. Accordingly, given the level of knowledge and skill in the art, an ordinary artisan would have been able to practice the invention without undue experimentation. In view of the foregoing, withdrawal of the rejection is respectfully requested.

Issues under 35 U.S.C. §103

Claims 1-8 stand rejected under 35 U.S.C. § 103(a) as allegedly obvious over PCT Publication No. WO 02/073181 to Hayade ("Hayade '181") or U.S. Publication No. 2005/0067278 to Sode, ("Sode"), in view of JP 2002-125689 to Hayade ("Hayade '689") and Oubrie *et al. Journal Biological Chemistry*, 2002, 277:3727-3732, ("Oubrie"), *see* Office Action, pages 9-11. Applicants respectfully traverse.

Basis for the rejection

According to the Examiner, Hayade '181 or Sode, which the U.S. national phase application of Hayade '181, describe an enzyme electrode comprising a chemically cross-linked enzyme/cytochrome for monitoring glucose levels. The Examiner states that the described enzyme is an *Acinetobacter calcoaceticus* derived PQQGDH and the cytochrome is cytochrome b562. The Examiner further states that Hayade '689 teaches the fusion of PQQGDH derived from *Acinetobacter calcoaceticus* and a biotin bound portion as a glucose sensor. Oubrie is cited for allegedly teaching that cytochrome b562, derived from *Comamonas testeroni*, functions as an electron acceptor of PQQGDH.

The Examiner admits that neither Hayade '181, Sode, Hayade '689, nor Oubrie describe combining glucose dehydrogenase and cytochrome by recombinant means to obtain a fusion protein. Nevertheless, the Examiner believes that an ordinary artisan, following the teachings of Hayade '181 or Sode regarding chemically combining PQQGDH from *Acinetobacter calcoaceticus* and cytochrome, would have recognized that the proteins could have been recombinantly expressed as a fusion protein. According to the Examiner, an ordinary artisan would have recognized that the biotin described in Hayade '689 could have been readily substituted with cytochrome c or b562, as described in Oubrie.

Standard for obviousness

When considering obviousness of a combination of known elements, the operative question is "whether the improvement is more than the predictable use of prior art elements according to their established functions." *KSR International v. Teleflex Inc.*, 82 USPQ2d 1385, 1396 (2007).

The claimed invention could not have been predictably achieved in view of the cited references

Initially, Applicants note that Hayade '181 was cited against claims directed to similar subject matter as the instant claims in corresponding British Application No. 0606955.3. The British patent was issued on February 27, 2008, as GB 2421951 B2, *see enclosed* Exhibit D1, *i.e.*, the July 20, 2007, response to the Hayade '181 rejection. Applicants submit that the issuance of a corresponding British patent is persuasive evidence that the claims are novel and non-obvious in view of the prior art.

Applicants further submit that an ordinary artisan could not have predictably achieved the instantly claimed fusion protein from the cited references. As noted above, Hayade '181 or Sode discloses a glucose sensor having PQQGDH co-immobilized on an electrode with cytochrome b562. Applicants submit that Hayade '181 or Sode merely describe a mixture of PQQGDH and cytochrome. As admitted by the Examiner, these references fail to teach or suggest a fusion protein of PQQGDH and cytochrome. Applicants further submit that an ordinary artisan would not have been motivated to prepare a fusion protein from the Hayde or Sode disclosure.

Further, it is unpredictable from the Hayade '181 or Sode references that the fusion protein described in the instant claims would have been functional. Applicants submit that an ordinary artisan could not have reasonably predicted whether or not the fusion protein could have been secreted in the periplasmic space of *E. coli* and undergone the proper post-translational modification, such that an electron generated from the reaction of PQQGDH with glucose could have been effectively transferred to the cytochrome c domain. In addition, an ordinary artisan could not have been reasonably certain from the teachings in Hayade '181 or Sode whether the electron, in turn, could have been effectively transferred to the electrode, such that a detectable signal could have been generated on the electrode.

Hayade '689 does not remedy the deficiencies of Hayade '181 or Sode. Hayade '689 discloses a fusion protein of PQQGDH and a biotin binding fragment. The fusion protein described in Hayade '689, accordingly, is totally unrelated to electron transfer. The Office Action states on page 11 that "[t]his may be easily accomplished by substituting the biotin in ... [the Hayade '689] fusion protein with cytochrome C or 562 and do so with a reasonable expectation of success." Applicants respectfully disagree. As discussed above, one could not have reasonably expected that a biotin fragment could have been substituted with a cytochrome c

or b562 to obtain a fusion protein exhibiting glucose dehydrogenase activity and electron transfer ability. Accordingly, the claimed fusion protein could not have been achieved with a reasonable expectation of success, based upon the disclosure of Hayade '689.

Oubrie also fails to remedy the deficiencies of Hayade '181, Sode, and Hayade '689. Oubrie, which discloses the crystal structure of a quino-hemo protein, alcohol dehydrogenase, allegedly "describes that cytochrome B562 derived from *Comamonas testeroni* has a function as an electron acceptor of pyrroloquinolinequinone glucose dehydrogenase", *see* Office Action, page 10. Applicants submit that this statement is without basis. Oubrie describes the structure of the cytochrome c domain of a quinohemoprotein alcohol dehydrogenase. Oubrie discloses NOTHING about cytochrome B562 or glucose dehydrogenase. Applicants submit that the assertion in the Office Action relies on a misunderstanding of the reference, and thus should be disregarded.

Moreover, Oubrie does not teach or suggest that the cytochrome c domain would be able to function as an electron transfer protein when separated from the alcohol dehydrogenase domain. Accordingly, a person of ordinary skill in the art at the time of the invention could not have easily conceived of the claimed fusion protein based upon the teachings in Oubrie or the other cited references.

Unexpected Effects

Furthermore, the claimed fusion protein results in remarkable effects, which could not have been expected from the cited references. For example, as shown in Fig. 5 of Hayade '181, PQQGDH and cytochrome b562, co-immobilized at a molar ratio of 1:1, showed much less response compared to the fusion protein of the instant invention. Hayade '181 shows in Fig. 4 that the current response, which is observed using a 100-fold molar excess of cytochrome b562 is increased, but little response is observed with a 1:1 molar ratio of Glucose Dehydrogenase/Cytochrome (GDH/Cyt) in the presence of a mediator. In the absence of a mediator, no current was observed with 1:1 molar ratio of GDH/Cyt, *see* Fig. 5 of Hayade '181. These results indicate that a significant amount of cytochrome is required for effecting electron transfer from GDH to cytochrome.

Similar results are also shown in two articles published after the priority date of Hayade '181 (but before the filing date of the instant application), which discusses electron transfer

between GDH and Cyt, *see* Exhibit D2, *i.e.*, Okuda *et al.*, *Anal. Lett.* 2002, 35, 1465, *enclosed.* Exhibit D2 discloses a glucose sensor with PQQGDH and cytochrome c or cytochrome b562 co-immobilized on an electrode. Figure 4 of Exhibit D2 shows the correlation between the current response and the molar ratio of GDH/Cyt, *see* page 1472 of Exhibit D2. The result demonstrates that 100-fold (at molar ratio) or more of cytochrome is required for providing a detectable response of the enzyme electrode. This result indicates that a significant amount of cytochrome is required for effecting electron transfer from GDH to cytochrome, even in the presence of an artificial electron mediator.

A subsequent report, demonstrates a direct electron transfer between PQQGDH and cytochrome b562, co-immobilized on an electrode tip, *see* Exhibit D3, *i.e.*, Okuda *et al.*, *Biosens. Bioeletron.*, May, 2003, 18, 699, 2003, enclosed,. Again, this system requires 100-fold or more molar excess of cytochrome b562.

In view of Hayade '181 and Exhibits D2 and D3, it was commonly known in the art at the time of filing that 100-fold or more molar excess of cytochrome is required for effecting electron transfer from PQQGDH to cytochrome when both enzymes are immobilized together on an electrode. A person of ordinary skill in the art, accordingly, could not have reasonably expected that the claimed fusion protein, which inevitably contains a 1:1 molar ratio of GDH and cytochrome, could provide a detectable current response. Therefore, the claimed fusion protein offers a significant improvement for use in a GDH-based glucose sensor.

In view of the foregoing, the claims are not rendered obvious by the cited references. Reconsideration and withdrawal is respectfully requested.

CONCLUSION

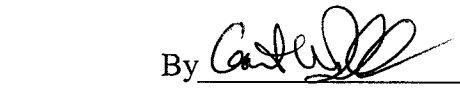
In view of the claim amendments, remarks and evidence submitted herewith, Applicants believe the present application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Linda T. Parker, Ph.D., Registration No. 46,046, at the telephone number of the undersigned below to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Director is hereby authorized in this, concurrent, and future replies to charge any fees required during the pendency of the above-identified application or credit any overpayment to Deposit Account No. 02-2448.

Dated: NOV 29 2010

Respectfully submitted,

By  GARTH M. DAHLEN
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Attachments

APPENDIX

List of Exhibits

Evidence in Support of the Claims under 35 U.S.C. §112, First Paragraph

US Publications:

Exhibit A1

US2003232418 A1 to Takeshima *et al.*, published December 18, 2003.

Exhibit A2

US2005260728 A1 to Suzumura *et al.*, published November 24.

Exhibit A3

US2007105173 A1 to Takeshima *et al.*, published May 10, 2007.

Foreign Patent Documents:

Exhibit B1

EP1167519A1 to K. Sode, published January 2, 2002.

Exhibit B2

EP1176202A1 to K. Sode, published January 30, 2002.

Exhibit B3

EP1369485A1 to Sode *et al.*, published December 10, 2003.

Exhibit B4

EP1437411A1 to K. Sode, published July 14, 2004.

Exhibit B5

EP1535996A1 to K. Sode, published June 1, 2005.

Exhibit B6

EP1544292A1 to K. Sode, published June 22, 2005.

Exhibit B7

WO0234919A1 to Kratzsch *et al.*, published May 2, 2002.

Exhibit B8

WO2006008132A1 to Boenitz-Dulat *et al.*, published January 26, 2006.

Exhibit B9

WO2006040172A1 to Boenitz-Dulat *et al.*, published April 20, 2006.

Exhibit B10

WO2007118647A1 to Boenitz-Dulat *et al.*, published October 25, 2007.

Scientific literature:

Exhibit C1

Igarashi, *et al.*, "Construction and Characterization of Mutant Water-Soluble PQQ Glucose Dehydrogenases with Altered Km Values—Site-Directed Mutagenesis Studies on the Putative Active Site, *Biochem. Biophys. Res.*, 1999, 264:820–824.

Exhibit C2

Sode *et al.*, "Increasing the thermal stability of the water-soluble pyrroloquinoline quinine glucose dehydrogenase by single amino acid replacement", *Enzyme and Microbial Technology*, 2000, 26:491-496.

Exhibit C3

Kojima *et al.*, The production of soluble pyrroloquinoline quinine glucose dehydrogenase by *Klebsiella pneumoniae*, the alternative host of PQQ enzymes, *Biotechnol. Lett.*, 2000, 22:343-1347.

Exhibit C4

Takahashi *et al.*, "Construction and Characterization of Glucose Enzyme Sensor Employing Engineered Water Soluble PQQ Glucose Dehydrogenase with Improved Thermal Stability", *Electrochemistry*, 2000, 68:907-911.

Exhibit C5

Yoshida *et al.*, "Secretion of water soluble pyrroloquinoline quinine glucose dehydrogenase by recombinant *Pichia pastoris*", *Enz. Microb. Technol.*, 2002, 30:312-318.

Exhibit C6

Sode *et al.*, "Construction of Engineered water-soluble PQQ Glucose Dehydrogenase with improved substrate specificity", *Biocatal. Biotransformation,* 2002, 20:405-412.

Exhibit C7

Igarashi and Sode, "Stabilization of Quaternary Structure of Water-Soluble Quinoprotein Glucose Dehydrogenase", *Mol. Biotechnol.*, 2003, 24:97-103.

Exhibit C8

Yoshida *et al.*, "Improved substrate specificity of water-soluble pyrroloquinoline quinine glucose dehydrogenase by a peptide ligand", *Biotechnol. Lett.*, 2003, 25:301-305.

Exhibit C9

Koh *et al.*, "Surface charge engineering of PQQ glucose dehydrogenase for downstream processing", *Biotechnology Lett.*, 2003, 25:1695–1701.

Exhibit C10

Okuda and Sode, "PQQ glucose dehydrogenase with novel electron transfer ability", *Biochem. Biophys. Res. Commun.*, 2004, 314:793–797.

Exhibit C11

Igarashi *et al.*, "Engineering PQQ glucose dehydrogenase with improved substrate specificity Site-directed mutagenesis studies on the active center of PQQ glucose dehydrogenase", *Biomol. Eng.*, April 2004, 21:81–89.

Exhibit C12

Okuda *et al.*, "Engineered PQQ Glucose Dehydrogenase-Based Enzyme Sensor for Continuous Glucose Monitoring", *Anal. Lett.*, 2004, 37:1847-1857.

Exhibit C13

Igarashi and Sode, "Construction and characterization of heterodimeric soluble quinoprotein glucose dehydrogenase", *J. Biochem. Biophys. Methods*, 2004, 61:331–338.

Exhibit C14

Tanaka *et al.*, "Increasing stability of water-soluble PQQ glucose dehydrogenase by increasing hydrophobic interaction at dimeric interface", *BMC Biochem.*, Feb. 16, 2005, 6:1-6.

Exhibit C15

Igarashi and Sode, "Protein Engineering of PQQ Glucose Dehydrogenase", Chapter 12 *in* Enzyme Functionality: Design, Engineering, and Screening, Marcel Dekker, Inc; New York; A. Svendsen, (ed.); ISBN: 0-8247-4709-7; 2004; 261-292.

Exhibit C16

Igarashi *et al.*, "Molecular engineering of PQQGDH and its applications", *Arch. Biochem. Biophys.*, August 1, 2004, 428:52–63.

Comparative evidence supporting unexpected effects of the claimed fusion protein

Exhibit D1

July 20, 2007, response submitted in British Patent Application No. 0606955.33

Exhibit D2

Okuda *et al.*, "The Application of Cytochromes as the Interface Molecule to Facilitate the Electron Transfer for PQQ Glucose Dehydrogenase Employing Mediator Type Glucose Sensor", *Analytical Letters*, 2002, 35:1465-1478.

Exhibit D3

Okuda *et al.*, "Glucose Enzyme Electrode using Cytochrome b_{562} as an electron mediator", *Biosensors and Bioelectronics*, 2003, 18:699-704.